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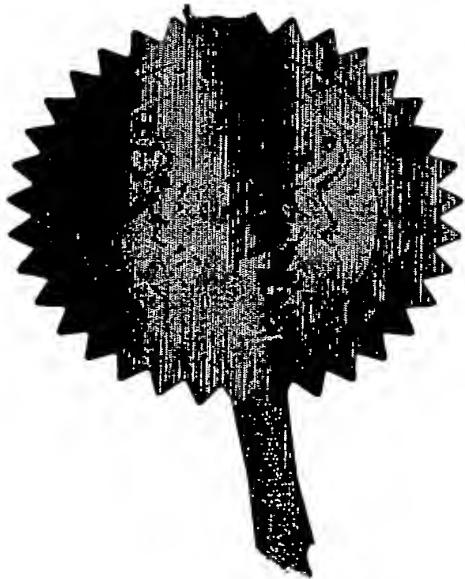
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25 JUL 03 E825392-1 D10098
1/7700 0.00-0317447.1**Request for grant of a patent**

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25 JUL 2003

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1. Your reference

10/705/P/GB

25 JUL 2003

2. Patent application number

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0317447.1

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)
 Tissuemed Limited
5 Killingbeck Drive
Leeds
LS14 6UF
Patents ADP number (*if you know it*)

7499346002

England

4. Title of the invention

Sheet or the like, for topical therapeutic use, and process for its preparation

5. Name of your agent (*if you have one*)
 "Address for service" in the United Kingdom
to which all correspondence should be sent
(*including the postcode*)

Adamson Jones

 Broadway Business Centre
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Number of earlier application

Date of filing
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Description 24

Claim(s) 0

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I/We request the grant of a patent on the basis of this application.

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Sheet or the like, for topical therapeutic use, and process for its preparationField of the Invention

5 This invention relates to a flexible multilamellar sheet, patch or film for topical application to internal and external surfaces of the body, for therapeutic purposes, to a process for the preparation of such products, and to methods of using such products. In particular, the invention relates to a self-adhesive, biocompatible and hydratable polymeric sheet with active constituents in powdered form applied to a
10 suitable support, which may be used for therapeutic purposes such as wound healing, joining, sealing and reinforcing weakened tissue, and for drug delivery, and to a process for preparing, and methods of using, such a sheet.

Background of the Invention

15 The use of therapeutic materials in the form of a sheet, patch or film, for topical administration to either internal or external organs of the body, is well documented for a wide range of medical applications. A disadvantage of products proposed hitherto, however, is that the degree of adhesion to the underlying tissue,
20 particularly in the longer term, may be inadequate. While the initial adhesion may be satisfactory, the sheet may subsequently become detached from the tissue, often after only a few seconds or minutes, eg as a result of hydration of the sheet following its application. In addition, the flexibility of the product may be insufficient for it to conform readily to the surface to which it is applied, which may
25 also have an adverse effect on its adhesion. As a result, it may be necessary to secure the product in place, eg through mechanical attachment using sutures, staples or the like. Alternatively, energy (eg light or heat energy) may be applied to the sheet in order to initiate chemical bonding of the sheet to the underlying tissue.
30 Clearly, such approaches suffer from drawbacks. The use of mechanical fastenings such as sutures or staples is often the very thing that the use of such

products is intended to replace or avoid. In many instances the use of such fastenings is either not wholly effective (eg on the lung), or undesirable as their introduction gives rise to further areas of tissue weakness. The use of external energy requires the provision and operation of a source of such energy. Such energy sources may be expensive and difficult to operate, particularly in the confines of an operating theatre or similar environment. Also, the use of external energy for attachment can be both time-consuming and (in some cases) requires significant careful judgement on the part of the surgeon, to evaluate when sufficient energy has been delivered to effect attachment without damaging the underlying tissue.

There have now been devised improvements to sheets or the like of the general type described above that overcome or substantially mitigate the above-mentioned and/or other disadvantages of the prior art.

15

Summary of the Invention

According to a first aspect of the present invention, there is provided a sheet having a multilayer structure, said structure comprising a core of a naturally occurring or synthetic polymeric material, the core being coated on at least one side thereof with a tissue-adhesive formulation comprising a naturally occurring or synthetic polymerisable and/or cross-linkable material in particulate form, the polymerisable and/or cross-linkable material being in admixture with particulate material comprising tissue-reactive functional groups.

25

In preferred embodiments of the invention, the tissue-adhesive formulation is applied to the core by mechanically compressing a blend of material containing tissue-reactive functional groups (hereinafter referred to as "tissue-reactive material") and the polymerisable and/or cross-linkable component, both in particulate form, onto one or both sides of the core.

The sheet according to the invention is advantageous primarily in that it bonds effectively to tissue, enabling it to be used in a variety of medical applications. The invention enables coating of the tissue-reactive materials onto (and into) a three-dimensional structural support, whilst maintaining the pliability and physical properties of the support. Furthermore, the adhesive performance of the tissue-reactive materials is not compromised when delivered to the target tissue in this form. Where, as in preferred embodiments, the support is perforated, the perforations provide a means of anchoring the tissue-reactive materials in the support. This reduces or eliminates cracking and crumbling of the tissue-reactive material as it is applied to the support (core), which would result in sub-optimal coverage of the target tissue, and thereby compromise the adhesive/sealant effects of the sheet.

The sheet exhibits good initial adhesion to the tissue to which it is applied (and may thus be described as "self-adhesive"), and furthermore remains well-adhered to the tissue over a longer timescale. Without wishing to be bound by any theory, it is believed that the initial adhesion of the sheet to the tissue is attributable to electronic bonding of the sheet to the tissue, and this is supplemented or replaced by chemical bonding between the tissue-reactive functional groups of the formulation and the tissue, in particular between amine and/or thiol groups on the tissue surface and the tissue-reactive groups of the sheet. Where the structural inner core of the device is perforated, channels can be created (during subsequent hydration following application) that offer a means of escape for fluid that may accumulate at the sheet/material interface. This feature eliminates a common problem associated with the use of adhesives within body cavities, namely over-hydration of the material by exudate, leading to a loss of contact.

The use of the sheet reduces or eliminates the need for additional means of mechanical attachment to the tissue (eg sutures or staples), or the need to provide external energy in the form of heat or light to bring about adherence of the sheet to the underlying tissue. Another advantage of the sheet according to the invention

is that it is applied to the tissue as a preformed article, rather than being prepared by mixing of materials immediately prior to use.

On contact with the moist tissue surface the formulation becomes hydrated,

5 thereby causing reaction between the tissue-reactive functional groups and the underlying tissue surface. Reaction may also take place between the tissue-reactive functional groups and the other components of the formulation to form a strong, flexible and tissue-adherent gel. This formulation thus absorbs physiological fluids (as a consequence of application onto exuding tissue surfaces), combined with additional solutions used to hydrate the formulation following application (the fluids can be commonly used solutions used in surgical irrigation) becoming gelatinous and adherent to the tissue surfaces, and thereby providing an adhesive sealant, haemostatic and pneumostatic function.

10

15 In addition, the reaction between the tissue-reactive functional groups and the underlying tissue results in high adhesion between the formulation and the tissue surface, and hence between tissues that are joined using the adhesive formulation. Furthermore, because the formulation is, until hydrated by contact with the moist tissue surface (and subsequent hydration), essentially inactive, the formulation is not prone to premature reaction and as a result the shelf-life of the sheet may be considerable.

20

By the term "sheet" is meant a three-dimensional article with a thickness that is considerably less than its other dimensions. Such an article may alternatively be described as a patch or a film.

According to another aspect of the invention, there is provided a method for the manufacture of a sheet according to the first aspect of the invention, which method comprises forming a core comprising naturally occurring or synthetic polymeric material, and coating at least one side of said core with a tissue-adhesive formulation comprising a naturally occurring or synthetic polymerisable and/or cross-linkable material in particulate form, the polymerisable and/or cross-linkable

25

30

material being in admixture with particulate material comprising tissue-reactive functional groups.

The invention also provides a method of joining a tissue surface to another tissue,
5 or of sealing a tissue surface, which method comprises applying to the tissue surface a sheet according to the first aspect of the invention.

Brief Description of the Drawings

10 Figure 1 is a schematic representation of the reaction between a tissue-reactive functional group (in the illustrated case an N-hydroxysuccinimide ester) and an amine-containing molecule such as a tissue protein.

15 Figure 2 shows the introduction of carboxyl group-bearing side chains into a poly(vinyl alcohol – vinyl acetate) copolymer.

Figure 3 represents the formation of a poly(N-vinyl-2-pyrrolidone-co-acrylic acid) copolymer.

20 Figure 4 shows the mechanism of free radical initiation of a polymerisation reaction.

Figure 5 illustrates the synthesis of a tissue reactive material.

25 Figure 6 is a schematic sectional view of a sheet according to the invention.

Detailed Description of the Invention

Nature of the core

The principal functions of the core are to provide the sheet with structural integrity and to provide a flexible substrate onto which the tissue-reactive formulation, in powder-form, can be applied.

- 5 The core can be prepared using any suitable polymeric material or combination of materials. The core may be biodegradable or non-biodegradable, and should be biocompatible, ie should be capable of application to tissues either within or external to the body without causing any immunological or other adverse reaction.
- 10 Examples of polymeric materials that may be used for the core are:

Polymers or co-polymers based on α -hydroxy acids, such as polylactide, polyglycolide, and also polycaprolactone and other polylactones such as butyro- and valerolactone.

15

Other examples may include:

- Alginates (ie polymers based on alginic acid, the polysaccharide obtained from seaweeds)
- 20 Polyhydroxyalkanoates
Polyamides
Polyethylene
Propylene glycol
Water-soluble glass fibre
- 25 Starch
Cellulose
Collagen
Pericardium
Albumin
- 30 Dacron
Polyurethane
Polypropylene

Polytetrafluoroethylene

The core may be prepared by casting, spinning or foaming of a solution of the polymeric material, or by moulding, weaving of filamentous material, or slicing from a block of material. Appropriate techniques for the preparation of the core by such methods will be familiar to those skilled in the art.

In preferred embodiments, the core is perforated after it has been formed, in order to create a web. Most preferably, the core is formed with a regular array of punctures, eg in a circular, square or hexagonal array.

Preferably, the punctures are between 50µm and 2mm in diameter and adjacent punctures are formed at a centre-to-centre separation of between 100µm and 5mm. Preferably, the punctures account for between 5% and 80% of the overall surface area of the core.

The core may have a thickness of from 0.005 to 5mm.

Composition of the coating formulation

The formulation with which the core is coated comprises a naturally occurring or synthetic polymerisable and/or cross-linkable material in particulate form, and a particulate material comprising tissue-reactive functional groups.

These two components may be blended in suitable proportions, which may depend on the particular materials used, as well as the desired properties of the resulting blend. Typically, the ratio of cross-linkable material to material comprising tissue-reactive functional groups will be between 0.2:1 and 10:1, more preferably between 0.5:1 and 10:1.

30

Application of the formulation to the core

The formulation may be applied to just one side of the core. More preferably, however, the formulation is applied to both sides of the core.

Where the core is perforated, application of the formulation to one or both sides of

5 the core causes the perforations to be filled with the formulation. In use, where the formulation is applied to both sides of a perforated core, the formulation that is present in the perforations effectively binds together the activated coatings on each side of the core, encapsulating the core between the two layers of activated coating.

10

The preferred method for applying the formulation to the core involves mechanically compressing (eg using a hydraulic press) a blend of the tissue-reactive material and the polymerisable and/or cross-linkable component, both in particulate form, onto one or both sides of the core.

15

The blend may be prepared by admixing particles of the tissue-reactive material with particles of the polymerisable and/or cross-linkable component.

20 The formulation may further comprise additional components such as structural polymers, surfactants, and plasticizers. Such further components may be present as discrete particles, or may be components of the particles of tissue-reactive material and/or polymerisable and/or cross-linkable component. In particular, the coating formulation may include filler materials, that may typically account for up to 50% by weight of the coating formulation. Examples of such materials include

25 cellulose derivatives (eg carboxymethyl cellulose, hydroxypropyl methylcellulose, etc), polyethylene glycol, polyvinylpyrrolidone and other commonly used pharmaceutical excipients.

30 The thickness of the coating applied to one or both sides of the core will typically be between 50µm and 0.5mm, more commonly from about 70µm to about 0.2mm.

Optionally, the surface of the sheet that, in use, is uppermost may be coated with a non-adhesive material. Most preferably, such a material is a synthetic polymer. Examples of suitable polymers include polyethylene glycols, polylactide and poly(lactide-co-glycolide). A sheet with such a non-adhesive coating will adhere 5 only to the target tissue (to which the underside of the sheet is applied) and not to surrounding tissues (eg the pleural or peritoneal wall). The non-adhesive coating may include a visibly-absorbing chromophore to enable identification of the non-tissue contacting surface of the sheet. An example of a suitable chromophore is methylthioninium chloride.

10

Nature of the particulate material comprising tissue-reactive functional groups

The particulate tissue-reactive material is preferably polymeric in nature. Most preferably, the polymer is a synthetic polymer.

15

By "tissue-reactive functional groups" is meant functional groups capable of reacting with other functional groups present in the tissue surface so as to form covalent bonds between the formulation and the tissue. Tissues generally consist partly of proteins, which commonly contain thiol and primary amine moieties.

20

Many functional groups such as imido ester, p-nitrophenyl carbonate, N-hydroxysuccinimide (NHS) ester, epoxide, isocyanate, acrylate, vinyl sulfone, orthopyridyl-disulfide, maleimide, aldehyde, iodoacetamide, and others, may react with thiols or primary amines, and therefore constitute "tissue-reactive functional groups".

25

Figure 1 illustrates the mechanism by which an NHS-functionalised polymer reacts with an amine-containing material such as a tissue protein represented by R-NH₂. The reaction is a nucleophilic displacement leading to the formation of an amide bond between the polymer and the tissue protein.

30

Tissue-reactive functional groups that may be of utility in the present invention are any functional groups capable of reaction (under the conditions prevalent when the

formulation is applied to tissue, ie in an aqueous environment and without the application of significant amounts of heat or other external energy) with functional groups present at the surface of the tissue. The latter class of functional group includes thiol and amine groups, and tissue-reactive functional groups therefore

5 include groups reactive to thiol and/or amine groups. Examples are:

imido ester
p-nitrophenyl carbonate
N-hydroxysuccinimide (NHS) ester
epoxide

10 isocyanate

acrylate

vinyl sulfone

orthopyridyl-disulfide

maleimide

15 aldehyde

iodoacetamide

N-hydroxysuccinimide (NHS) ester is a particularly preferred tissue-reactive functional group.

20

In general, the tissue-reactive material may be formed by derivatisation of a suitable polymer precursor. Classes of polymer which lend themselves to such derivatisation include those that contain carboxylic acid or alcohol functional groups, or related structures. Polymers that may be used include polymers that 25 are commercially available or polymers that are prepared specifically for this purpose. Naturally-occurring materials such as sucrose or a derivatised cellulose may also be used.

Commercially available polymers that may be used include polyvinylalcohol (PVA).
30 In the case of PVA, the functional groups may be introduced by first adding a chain extending or linking group, for example an acid functionality that can be further reacted with N-hydroxy succinimide. Figure 2 shows the addition of a

chain-extending group to a copolymer of vinyl acetate and vinyl alcohol, the chain-extending group terminating in a carboxylic acid group that may be converted to the corresponding NHS-ester. The copolymer starting material (in which molar fraction x of vinyl alcohol groups may be 0.85-0.995) is reacted with a cyclic

5 anhydride (in the example illustrated, succinic anhydride) in the presence of a base such as pyridine. Between 5% and 40% of the alcohol groups are derivatised to form the carboxylic acid-bearing side chains (ie $a+b=x$, with a being between 0.05x and 0.40x), which may then be converted to the NHS-ester by conventional methods that are known per se.

10

Where the polymer support is synthesized for the purpose of subsequent derivatization, a wide variety of monomers may be used. Examples include *N*-vinyl-2-pyrrolidone, acrylic acid, vinyl acetate, vinyl acetic acid, mono-2-(methacryloyloxy)ethyl succinate, methacrylic acid, 2-hydroxyethyl methacrylate,

15 2-hydroxypropyl methacrylate, (polyethylene glycol) methacrylate or other monomers containing acid or alcohol functionality. Such monomers may be polymerised via various standard polymerisation techniques, including free radical techniques using an initiator such as benzoyl peroxide, 2,2'-azobisisobutyronitrile (AIBN), lauroyl peroxide, peracetic acid etc. One preferred example of such a
20 polymer is poly(*N*-vinyl-2-pyrrolidone-co-acrylic acid) co-polymer polymerised using AIBN as initiator. The polymerization of this material is illustrated in Figure 3, in which the molar ratio of acrylic acid-derived units may be between 0 and 1.0, preferably less than 0.60, and most preferably between 0.025 and 0.25. The copolymer may be further reacted with *N*-hydroxysuccinimide to form the
25 tissue-reactive material.

Where, as is preferred, the tissue-reactive functional groups are NHS-esters, at least one of the monomers used in the preparation of the tissue-reactive material must contain a carboxylic acid group or a group capable of being reacted with
30 another material to form an acid functionality.

In the preferred case in which the tissue-reactive material is an NHS ester of poly(N-vinyl-2-pyrrolidone-co-acrylic acid) copolymer (PVP-co-PAA), the molar ratio of acrylic acid-derived units is preferably between 0.05 and 0.50; and hence that of the vinyl pyrrolidone-derived units is between 0.50 and 0.95.

5

The acrylic acid groups are preferably derivatised with NHS groups. A copolymer of PVP and PAA, in which the carboxyl groups of the acrylic acid-derived units carry NHS groups, is referred to herein as NHS-activated PVP-co-PAA.

10 As noted above, the activity of the tissue-reactive material (ie the degree to which the tissue-reactive functional groups of that material bind to the tissue) may be controlled by varying the proportion of that material in the formulation. The concentration of the tissue-reactive material in the formulation may be varied quite widely, eg from 10% w/w or less up to 50% w/w or more.

15

The formulation may contain one type of tissue-reactive material, or more than one type of tissue-reactive material.

20 Preferably, all or substantially all of the available sites in the precursor to the tissue-reactive material will be derivatised (ie the tissue-reactive functional groups will be introduced into all or substantially all of the available sites in the precursor to the tissue-reactive material). The degree of binding between the tissue-reactive functional groups and the tissue to which the formulation is applied will then be a function of the amount of tissue-reactive material in the formulation.

25

Nature of the polymerisable and/or cross-linkable component

30 The polymerisable and/or cross-linkable component of the formulation may be naturally occurring or synthetic, eg selected from polysaccharides, polylactates, polyols and proteins, and derivatives thereof. The polymerisable component may be partially or fully cross-linked.

Proteins are preferred materials for the polymerisable and/or cross-linkable component of the formulation because they are rich in functional groups that are reactive to tissue-reactive functional groups. Hence, the tissue-reactive functional groups will react not only with the tissue surface to which the formulation is

5 applied, but also with the polymerisable and/or cross-linkable component of the formulation.

A particularly preferred protein for use in the invention is albumin, particularly mammalian albumin such as porcine, bovine or human albumin.

10 Preferred synthetic polymers that may be, or may be present in, the polymerisable and/or cross-linkable component of the formulation include multifunctionally activated synthetic polymers, ie synthetic polymers that have, or have been chemically modified to have, a plurality of functional groups that are capable of reacting with each other or with other functional groups present in the formulation to form covalent bonds. Preferred multifunctionally activated synthetic polymers include chemically modified polyalkylene glycols, in particular polyalkylene glycols that have been modified to contain multiple primary amino or thiol groups.

15

20 Suitable modified polyalkylene glycols include both linear and branched ((et so-called "3-arm" and "4-arm") compounds. Examples of suitable multi-amino polymers are those sold under the trade name JEFFAMINE. These are based on backbones of polyethylene glycol and/or polypropylene glycol units with terminal amine groups. Methods by which similar and analogous chemically modified polymers can be prepared will be readily apparent to those skilled in the art.

25

Manufacture of the components of the formulation

30 The particulate tissue-reactive material and the particulate polymerisable and/or cross-linkable component may be prepared by any suitable means. Particularly where the latter component is proteinaceous, the particles are preferably prepared by freeze- or heat-drying of an aqueous solution or suspension. To enhance

subsequent reaction of the proteinaceous material with the tissue-reactive material, the solution or suspension is preferably buffered to an alkaline pH prior to drying.

5 *Physical form of the sheet*

The sheet may typically have an overall thickness of from 0.05 to 10 mm, typically 0.05 to 2mm, and more commonly 0.05 to 0.5 mm, eg about 200 μ m or 300 μ m or 400 μ m.

10

The sheet may be produced with, or subsequently cut to, dimensions of from a few square millimetres up to several tens of square centimetres.

Therapeutic applications of the sheet

15

The sheet according to the invention is suitable for application to both internal and external surfaces of the body, ie it may be applied topically to the exterior of the body (eg to the skin) or to internal surfaces such as surfaces of internal organs exposed during surgical procedures.

20

The sheet according to the invention is particularly suitable for surgical applications in the following areas:

Thoracic / cardiovascular

25 General surgery

ENT

Urology

Oral / maxillofacial

Orthopaedic

30 Neurological

Gastroenterology

Ophthalmology

Gynaecology / obstetrics

Possible uses are described in more detail below.

5 *Wound healing*

The degradable nature of the sheet means that it may support and promote wound healing both during internal and topical procedures. Once the sheet begins to degrade fibroblasts will move in and begin to deposit components of the extracellular matrix. The sheet therefore can be used as an internal or external dressing. In addition, factors such as growth factors and cAMP that are known to promote the proliferation of skin cells may be added to the sheet to assist in the healing process. The sheet may be designed to control the transmission of moisture and infectious agents, and thus be useful particularly in the treatment of burns.

15

Skin closure

The sheet may be applied topically to promote wound closure (as an alternative to sutures). This may have beneficial effects in that it may reduce scarring, and the sheet may thus be useful for cosmetic purposes during minor surgery (eg in Accident and Emergency Departments). The self-adhesive properties of the sheet make it easy to apply quickly.

Hernia repair

The sheet may be used to provide reinforcement in hernia repair procedures. The self-adhesive attachment overcomes the potential issues faced by conventional surgical reinforcing mesh products, which require suturing or stapling in an already weakened area. The sheet for such a procedure may be engineered to have short or long term durability, depending on the degree of tissue repair required. The sheet may also be able to withstand the application of staples.

30

Anastomosis

The self-adhesive sheet according to the invention provides a means of rapid sealing of, and prevention of leaks in, joined tubular structures such as blood vessels, and vascular and bladder grafts, and the GI tract. The ability of the sheet

5 to support tissue repair may be of particular value if used in nerve repair.

Sealing large areas of tissue

The good sealing and handling properties of the sheet, combined with its self-adhesive properties and ability to be manufactured to cover a large surface area,

10 mean that it may be of particular use in sealing resected tissue surfaces – in particular those where diffuse bleeding is an issue (eg the liver). The sheet also provides an ideal support matrix for tissue repair at such sites. This could also be applicable to limiting leakage of cerebro-spinal fluid following neurological surgery.

15 *Sealing air leaks*

In addition to the sheet properties described above, the high tensile strength and good inherent elasticity of the sheet, make it particularly suitable for sealing air leaks in the lung, particularly following lung resection. Again, after effecting a seal, the sheet provides an ideal support matrix for tissue repair at such sites.

20

Haemostasis

The sheet may be applied to a bleeding area, acting as a physical barrier. The tissue-reactive polymers in the formulation may immobilise proteins and thereby promote haemostasis.

25

Therapeutic agent administration

Drugs and other therapeutic agents (including biologically active agents such as growth factors, and even cells and cellular components) may be added to solution(s) used to form the components of the sheet, or covalently linked to

30 components prior to their use in sheet formation. Once the sheet is in place, following application to the desired site, the drug will be slowly released, either by diffusion out of the sheet, or by engineering the sheet so that as it degrades over

time the drug is released. The rate of release can be controlled by appropriate design of the sheet. The sheet thus provides a means of delivering a known amount of drug either systemically or to a precise locus.

5 *Prevention of Post-Surgical Adhesions*

Post-surgical adhesion, the formation of undesired connective tissue between adjacent tissues, is a serious problem which can give rise to major post-surgical complications. It is a particular problem in bowel surgery where it can cause, for instance, twisting of the bowel, which may then necessitate further surgical intervention. The application of sheet material having self-adhesive properties in accordance with the invention to tissues exposed in a surgical procedure can be effective in preventing post-surgical adhesions between that tissue and neighbouring tissues.

15 Detailed Description of Preferred Embodiments

The invention will now be described in greater detail, by way of illustration only, with reference to the following Examples.

20 Example 1

Synthesis of NHS-activated PVP-co-PAA

(a) Polymerisation of acrylic acid and N-vinyl-2-pyrrolidone

25 The polymer is formed via the polymerisation of monomers such as N-vinyl-2-pyrrolidone and acrylic acid, as shown in Figure 3.

A number of methods may be used to initiate the polymerisation, such as free radical, ionic (cationic or anionic), thermal, UV, redox etc. Free radical polymerisation is the preferred polymerisation method and 2-2'-azo-bis-isobutyrylnitrile (AIBN) is the preferred initiator. The AIBN decomposes into two

radicals which can then attack the carbon-carbon double bond in the vinylic monomer (acrylic acid) as shown in Figure 4.

This will continue until termination of chain growth, via combination,

- 5 disproportionation etc.

The reaction solvent may be N,N'-dimethylformamide, toluene, or any other suitable solvent with a boiling point greater than 100°C. Toluene is the currently preferred solvent.

10

A typical polymerisation method is as follows:

Solvent is charged to the reaction flask, usually around 5-10ml of solvent per gram of monomer used is sufficient. The solvent is heated in an oil bath to a

- 15 temperature sufficient for the generation of free radicals from the chosen initiator, 80-85°C is the optimum temperature when using AIBN as the initiator. Oxygen-free nitrogen is bubbled through the solvent to remove any dissolved oxygen. Oxygen is also removed from the monomers in the same manner. The initiator is added to the solvent and allowed to dissolve. The monomers are added and the
- 20 vessel closed. A nitrogen inlet and an escape needle may also be used.

The reaction is allowed to stand for around 18 hours. The reaction mixture is cooled and the polymer is isolated from the solvent/polymer solution by precipitation in 5:1 hexane/isopropanol followed by filtration. Successive washes

- 25 with diethyl ether are required to remove all traces of polymerisation solvent from the polymer. After 4-5 diethyl ether washes the polymer is dried under reduced pressure to constant weight.

Typical reaction conditions are shown in Table I:

Table 1

Solvent (vol)	Monomer (g)	AIBN (g)	T (°C)	Time (hrs)	Yield	Mn	Mw	Polydispersity Index
Toluene (100ml)	1.5 (20mol%)	8.5 (80mol%)	0.02 (0.125%)	80	3	-	-	-
Toluene (100ml)	0.7 (10mol%)	9.3 (90mol%)	0.02 (0.125%)	80	3	54%	80040	38800
Toluene (100ml)	0.7 (10mol%)	9.3 (90mol%)	0.04 (0.25%)	80	3	58%	74240	38340
DMF (100ml)	0.7 (10mol%)	9.3 (90mol%)	0.02 (0.125%)	80	3	62%	54000	25150
Toluene (100ml)	0.5 (7.5mol%)	9.5 (92.5mol%)	0.02 (0.125%)	80	3	-	-	-
Toluene (100ml)	0.35 (5mol%)	9.65 (95mol%)	0.2 (0.125%)	80	3	-	-	-

(b) Reaction of PVP-co-PAA and N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide

NHS-activated PVP-co-PAA is formed from the reaction of PVP-co-PAA and N-

5 hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (Figure 5).

10g of PVP-co-PAA containing 0.094 moles of acrylic acid repeat units is dissolved in 50 ml of dried N,N'-dimethylformamide by stirring in a dry 100ml round bottomed flask. 0.01 moles of N-hydroxysuccinimde (1.15g) is added to the 10 polymer solution and is allowed to dissolve.

Dicyclohexylcarbodiimide (2.06g) is melted in an oven at 60°C and added to the polymer solution. This is left to stir at room temperature for at least 24 hours. The formation of a white precipitate (dicyclohexylurea) is observed. After 24 hours the 15 precipitate is removed by filtration, and the flask and filter washed with a small amount of dry DMF. The polymer is isolated by precipitation in 5:1 hexane/iso-propanol and filtration. The polymer is further purified by repeated washes with dry diethyl ether. The yield is between 50-70%.

20 Example 2

Blending of NHS-activated PVP-co-PAA with freeze-dried albumin

a) Powders of NHS-activated PVP₈₀-co-PAA₂₀ copolymers have been blended (1:1) with freeze dried porcine albumin (Sigma Aldrich; previously buffered to pH 25 10.5).

b) Powders of NHS-activated PVP₇₀-co-PAA₃₀ copolymers have been blended (1:1) with freeze-dried human albumin (Baxter human albumin infusion (20%) 30 previously buffered 1:1 with to pH 10.5 buffer).

c) Powders of NHS-activated PVP₇₀-co-PAA₃₀ copolymers have been blended (2:1) with freeze dried porcine albumin (previously buffered to pH 10.5 buffer).

Example 3

Blending of excipients with powdered PVP(NHS)₂₀ and freeze-dried porcine albumin previously buffered to pH 10.5 (PSA)

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Powders of PVP(NHS)₂₀ and PSA (1:1) have been blended with excipients such as hydroxypropyl cellulose, poly(vinyl pyrrolidone) and microcrystalline cellulose.

Example 4

10 **Preparation of perforated poly(lactide-co-glycolide) (PLG) film**

A PLG film, approx 50-100µm thick is placed between two blocks of perspex or other sheet material. The perspex blocks have a series of pre-drilled holes in registration with each other. A syringe needle or the like is pushed through the 15 holes in the perspex blocks, thereby puncturing holes in the PLG film. The needle is withdrawn, leaving a hole in the film. The process is repeated until a series of holes of equal diameter and equal spacing have been punctured in the film. The film is released from between the two blocks of perspex.

20 **Example 5**

Application of coating formulation to the film

The perforated PLG film prepared in accordance with Example 4 is cut to the desired shape using a suitable 13mm diameter punch. Into a suitable tablet die, 25 15mg of the PVP(NHS):HSA co-powder (4:1) is loaded evenly onto the bottom surface ensuring an even distribution of material. The pre-cut perforated PLG film is placed on top of this semi-compressed powder layer ensuring close contact between the film and the powder beneath. Another layer of powder (15mg; same composition as the first layer) is loaded onto the PLG film and distributed evenly. 30 The tablet die is loaded into a hydraulic press and the film/powder composite is compressed at a load of less than 0.5 tonne.

Example 6Application of sheet to tissue

The tissue surface is prepared in accordance with conventional surgical technique.

5 The sheet is applied onto (and if necessary) around the tissue surface with moderate pressure to ensure satisfactory contact to the tissue. Following application, the sheet is hydrated with saline.

Example 7Measurement of Adhesive Strength

A Universal testing machine (UTM, Zwick/Roell BZ2,5) was used to test the adhesive strength of test materials to freshly excised liver or lung tissue. Details of the testing procedure are summarised below.

15 A small section of tissue (4 cm × 4 cm × 1 cm (depth)) was prepared and mounted into a purpose-made holder at the base of the test machine. The surface of the tissue was sprayed with saline. The test specimen (with sample holder attached to enable subsequent removal) was placed onto the tissue surface with a moderate force to ensure full contact. The material was left on the tissue for 5 minutes and then wholly submerged in saline for a further 5 minutes. Whilst holding the tissue in place using a suitable clamp the folded tip of the sample holder was inserted in the grips of the UTM. The sample was positioned appropriately to ensure that the sample was aligned with the grips. The grip was then moved at 180° from the test sample thereby removing the sample from the tissue. The UTM software (Zwick TestXpert ver 9.0) can be used to calculate the energy of adhesion (mJ) of the test material. Adhesion tests were performed on powder compositions of differing compositions and compared to a commercially available polyethylene glycol (PEG) based sealant.

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Table II shows the data obtained by testing compressed films as a function of composition.

Table II

Test medium Substrate	Tissue reactive material	Crosslinkable material	Ratio of components	Mean energy of adhesion (SD)/ mJ (n=6)
Porcine liver	PVP(NHS)	Human albumin	4:1	1.3 (0.29)
Porcine liver	PVP(NHS)	Human albumin	1:4	1.0 (0.43)
Porcine liver	PVP(NHS)	Human albumin	1:1	1.0 (0.27)

5 The results listed in Table II demonstrate the adhesion performance of the co-powder formulations of PVP(NHS)₂₀ and freeze dried human albumin.

In a further study, Table III illustrates the results of adhesion testing of sheet formulations, (13mm diameter circular discs) studied as a function of the number 10 of perforations in a PLG film with active co-powders pressed into either side of the PLG film. The powders utilised were PVP(NHS)₂₀ and human albumin in a 4:1 ratio.

Table III

15

Test medium	No of perforations in 13mm diameter sheet.	Mean energy of adhesion (SD)/ mJ (n=6)
Porcine liver	0	0.39 (0.14)
Porcine liver	10	0.75 (0.25)
Porcine liver	20	1.14 (0.37)

The results listed in Table III demonstrate that the energy of adhesion of multi layered sheets is proportional to the number of perforations in the PLG inner film.

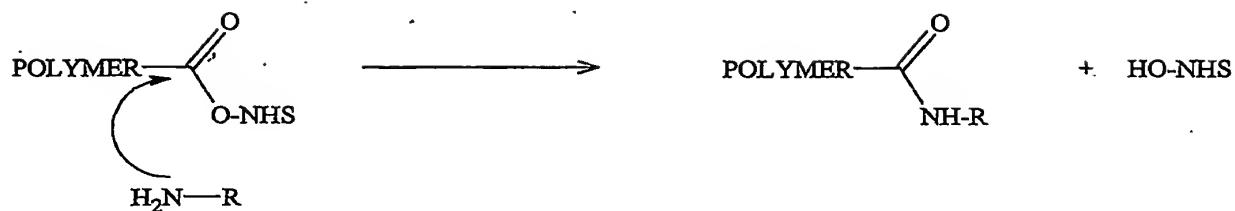
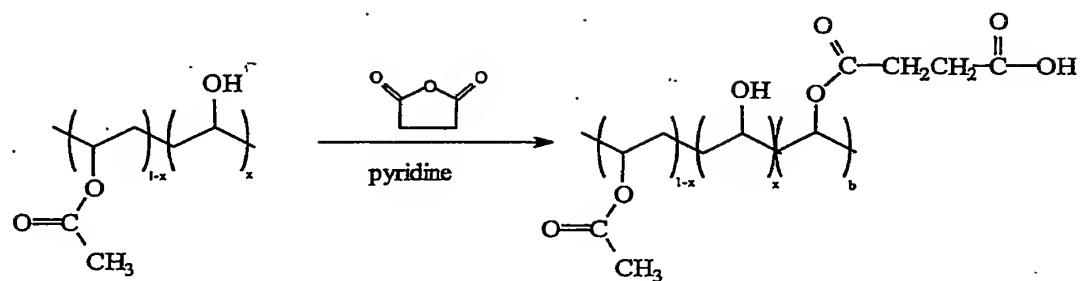
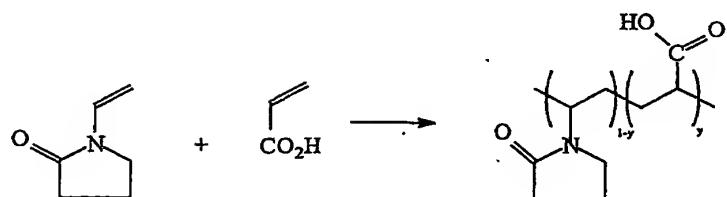
Furthermore, the work reveals that all three formulations evaluated offered greater adhesion than a commercially available polyethylene glycol (PEG) based sealant (see table 2).

5 Example 8

Schematic representation of a sheet according to the invention

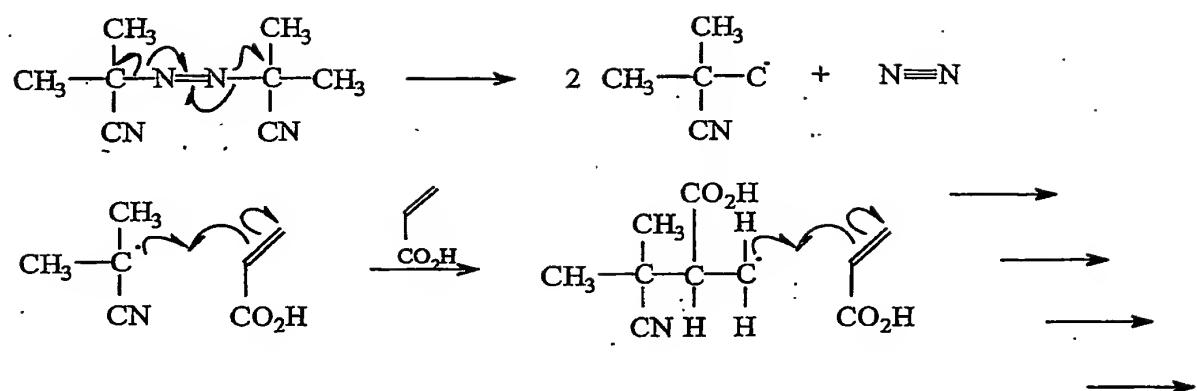
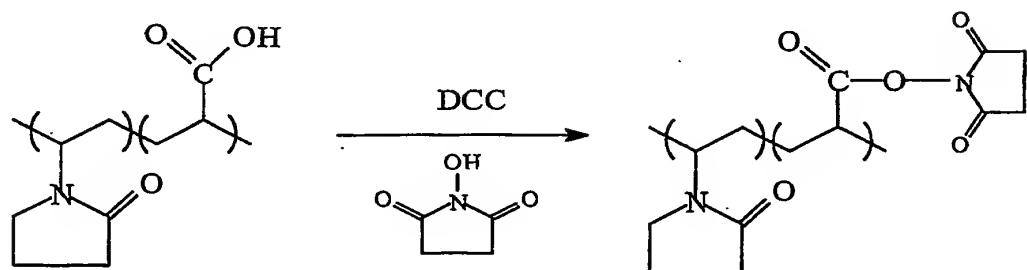
Figure 6 shows (schematically and not to scale) the structure of a typical sheet prepared in accordance with the invention. The sheet comprises a core in the form of a film 1 of PLG which has a regular array of perforations. Layers 2,3 of a tissue-reactive formulation are pressed onto both sides of the film 1 such that the tissue-reactive formulation penetrates into, and fills, the perforations. Finally, a non-adhesive layer 4, again of PLG, is applied to one surface of the sheet. The non-adhesive layer 4 may include a chromophore that gives the non-adhesive surface a discernible colour, thereby identifying that surface (and hence indicating which side of the sheet is to be applied to the tissue).

1/3

Figure 1Figure 2Figure 3

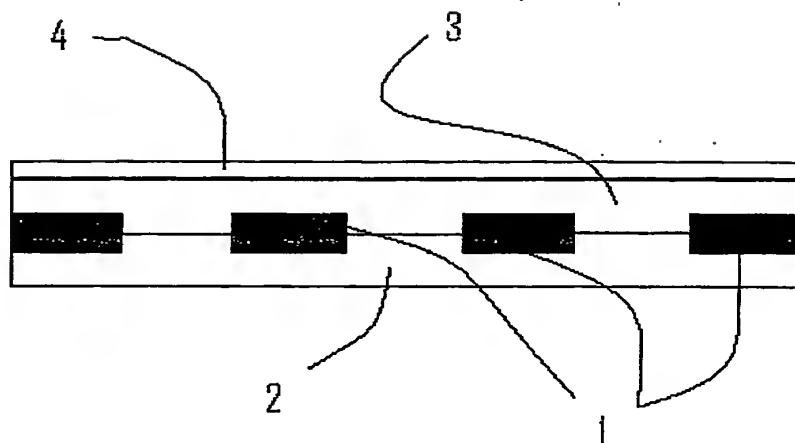
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Figure 4Figure 5

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Figure 6



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